Sex Steroid Hormones in Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** To investigate the relationships between concentrations of sex hormones and measures of disease activity in patients with primary Sjögren's Syndrome (pSS).

Methods. Fifty-four women were evaluated: 39 patients (age, Q1,Q3: 57.0 yrs; 46, 66) diagnosed with pSS and 15 patients (49.0 yrs; 45, 60) who did not meet diagnostic criteria for pSS. The following measures of disease activity were assessed: serological data [antinuclear antibody, rheumatoid factor, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum immunoglobulin levels (IgG, IgA, IgM), serum protein, anti-SSA, and anti-SSB], labial minor salivary gland focus score, salivary flow rates, and objective measures of eye dryness (fluorescein corneal staining and unstimulated Schirmer's I test). Spearman correlations were calculated between these indices of disease activity and serum levels of sex hormones: dehydroepiandrosterone (DHEA), DHEA sulfate, androstenedione, testosterone, dihydrotestosterone (DHT), estrone, estradiol, and sex hormone binding globulin (SHBG).

Results. Numerous differences were noted between cases and controls with disease activity measures. All median values of sex steroid hormones were within the range of normal for pSS cases. Positive correlations were noted between testosterone and ESR ($r = 0.36$, $p = 0.03$), testosterone and serum protein ($r = 0.37$, $p = 0.05$), and testosterone and focus score ($r = 0.44$, $p = 0.007$). Negative correlations were present between SHBG and anti-SSA ($r = –0.33$, $p = 0.05$), SHBG and anti-SSB ($r = –0.43$, $p = 0.009$), and DHT and CRP ($r = –0.41$, $p = 0.05$). No correlations were noted between estrogens and measures of pSS disease activity.

Conclusion. Higher levels of disease activity (ESR, serum protein, and focus score) were associated with higher concentrations of testosterone. No correlation between disease activity and estrogens was found. (J Rheumatol 2003;30:1267–71)

Key Indexing Terms:
SJÖGREN'S SYNDROME ANDROGENS ESTROGENS
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Women have a higher incidence of autoimmune disease, including Sjögren's syndrome (SS), which has a 9:1 female predominance[1,2]. The gender disparity present in autoimmune disease suggests that sex hormones may play a role in the etiology and/or disease progression.

One possible factor influencing the disproportionate gender prevalence for numerous autoimmune diseases may be related to women generally having more pronounced immunological responses[3,4]. More specifically, animal models of SS suggest estrogens may aggravate disease activity, while androgens are protective[5,6].

Androgens have been shown to be of benefit in SS mouse models. For example, Sullivan, *et al* demonstrated a therapeutic benefit of testosterone in decreasing lacrimal gland lymphocytic infiltrate and function[6]. Testosterone has also been beneficial in decreasing proinflammatory cytokines and suppressing autoimmunity in SS animal models[5,7].

While animal models have shown an association of estrogens and disease activity, evidence in humans suggests that increasing estrogen levels may actually aggravate symptoms associated with SS. A recent epidemiologic study by Schaumberg, *et al* showed an increased risk of dry eye syndrome with hormone replacement therapy[8]. Additionally, a possible link with estrogen therapy and the onset of SS has been suggested in a case report[9].

The concentration and significance of sex hormones in patients with SS have not been well characterized. Mouse models of SS and recent human studies suggest estrogens may enhance and androgens may suppress autoimmune disease. We compared sex steroid hormone concentrations between cases of primary SS (pSS) and controls, and investigated if any relationships exist between these hormones and measures of disease activity in patients with pSS. We

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hypothesized that patients with pSS would have lower concentrations of androgens than controls, and that androgens would be negatively correlated with disease activity. Further, we hypothesized that estrogens would be elevated in patients with pSS and be positively correlated with disease activity.

MATERIALS AND METHODS

Patient selection. A total of 54 women were evaluated for pSS at the Sjögren's Syndrome Clinic at the National Institutes of Health, Bethesda, MD. Two sets of patients were evaluated for sex steroid hormone information: 22 pSS patients at their baseline visit for a dehydroepiandrosterone (DHEA) drug trial and 32 consecutive prospective xerostomic patients being evaluated to determine if they had pSS. Classification by the San Diego criteria resulted in 39 pSS cases and 15 non-pSS controls[10]. For further analysis the entire population was also classified by the European Classification criteria[11].

Data collection. Diagnostic and laboratory measures for these patients were collected prospectively. These studies were approved by the National Institute of Dental and Craniofacial Research (NIDCR) Institutional Review Board.

The following diagnostic tests and clinical information were collected: (1) demographic information (age, race, sex); (2) degree of lymphocytic infiltration of the minor salivary gland biopsy (focus score)[12]; (3) objective ophthalmologic findings including presence of staining with ocular dyes (van Bijsterveld score) and Schirmer's I test without anesthesia measured in mm/5 min[11,13]; (4) serum IgG, IgA, IgM (mg/dl), anti-SSA and anti-SSB determined by ELISA kit (Hemagen Diagnostics, Columbia, MD, USA), antinuclear antibody (ANA) titer by slide test using HEp-2000 substrate (Immuno Concepts, Mt. Laurel, NJ, USA), rheumatoid factor (RF; IU/ml) by nephelometry, Westergren erythrocyte sedimentation rate (ESR), and serum protein (g/dl); (5) salivary flow rates (unstimulated and 2% citric-acid stimulated parotid and submandibular flow) collected individually from the major salivary glands using established techniques[14]; (6) sex steroid hormone levels including DHEA by radioimmunoassay, DHEA sulfate by chemiluminescent assay, androstenedione by radioimmunoassay, total testosterone by chemiluminometric immunoassay, dihydrotestosterone (DHT) by radioimmunoassay, sex hormone binding globulin (SHBG) by 2-site chemiluminescent immunoassay, estrone by radioimmunoassay, and estradiol by chemiluminometric immunoassay; and (7) use of hormone replacement therapy (HRT).

A total of 54 patients were evaluated for pSS applying the San Diego criteria[10]. Cases of pSS were confirmed when all 3 of the following diagnostic tests were positive: (1) positive lip biopsy (focus score > 1); (2) positive objective eye finding (Schirmer's ≤ 5 mm or fluorescein corneal staining; van Bijsterveld score (≥ 4); and (3) positive laboratory value (elevated immunoglobulin, ANA, anti-SSA, anti-SSB, or RF). The entire population was also classified by the European classification criteria into meeting versus not meeting the criteria[11]. No patient was taking DHEA as an over-the-counter supplement at the time of screening.

Statistical analysis. The Wilcoxon rank sum test was used to compare continuous variables. Chi-square tests were used for dichotomous variables. A univariate analysis with Spearman correlations was only done for pSS patients for measures of disease activity with sex steroid hormones. The SAS statistical program (SAS Institute Inc., Cary, NC, USA) was used for analysis.

RESULTS

Of the 54 patients evaluated, 39 were identified as pSS cases and 15 as non-pSS controls. A comparison of demographic and SS disease activity measures is shown in Table 1. The only difference in sex steroid hormones identified between cases and controls was in DHT, which was decreased in the non-pSS controls (p = 0.04) (Table 2). However, all median values of sex steroid hormones were within the range of normal for pSS cases. For controls, the median value of SHBG was just above the normal range and DHT was below the normal range.

In examining the relationship between hormone levels and disease measures, positive correlations were noted between testosterone and the following factors: ESR (r = 0.36, p = 0.03), serum protein (r = 0.37, p = 0.05), and focus score (r = 0.44, p = 0.007) in patients with pSS. Negative correlations were present between SHBG and anti-SSA (r = –0.33, p = 0.05), SHBG and anti-SSB (r = –0.43, p = 0.009), and between DHT and CRP (r = –0.35, p = 0.04). No correlations were noted between estrogens and measures of pSS disease activity. Age revealed negative correlations with androstenedione (r = –0.34, p = 0.04). Age also had negative correlations with DHEA, DHEA sulfate, DHT, and estradiol (r = –0.47 to –0.54, p = 0.003 to 0.001) and was not correlated with testosterone or estrone levels.

When we compared sex steroid hormone findings in cases and controls categorized utilizing the European Classification Criteria[11], similar results were seen: positive correlations between testosterone and RF, protein and focus score, and between DHT and RF. Negative correlations were found between SHBG and anti-SSA and SHBG and anti-SSB. Using this diagnostic set, the number of patients was increased by 7. No differences in any sex steroid hormones between cases (n = 46) and controls (n = 8) were identified with the European Classification Criteria. This may be partly related to the small number of controls for comparison.

HRT was used by 12/39 pSS cases and 7/15 controls. When pSS patients and controls taking HRT were not included, median concentrations of sex steroid hormones were within the normal range with the exception of a low median DHT (0 ng/dl) in the xerostomic controls, which was similar to the main analysis. No statistical differences were present in sex steroid hormone concentration between pSS and xerostomic controls not taking HRT.

A comparison of pSS patients taking HRT to those not taking HRT showed significant differences in SHBG and estrone. Patients using HRT had higher median levels of SHBG than pSS patients not taking HRT (116 vs 75 nmol/l; p = 0.006). Similarly, median estrone levels were higher for patients taking HRT versus those not on HRT (120.5 vs 24.5 pg/ml; p < 0.0001). No significant difference was noted for any measure of pSS disease activity between patients using HRT and those not using HRT.

When the analysis of pSS excluded HRT, the following correlations remained: DHT and CRP (r = –0.41, p = 0.05) and SHBG and anti-SSB (r = –0.43, p = 0.03). Other significant correlations appeared, including a positive correlation between testosterone and fluorescein staining (r = 0.47, p =

Table 1. Comparison of demographic and disease activity measures for cases with primary SS and controls. For most measures the median and 25th to 75th percentile values (Q1–Q3) are shown. For the remaining measures the percentage of positive individuals is given.

Measure	Cases (pSS)*, n = 39	Controls, n = 15	p
Age	57.0 (46, 66)	49.0 (45, 60)	NS
Focus score	8 (5, 12)	1 (1, 3)	< 0.0001
Unstimulated parotid, ml/min/gland	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	NS
Stimulated parotid, ml/min/gland	0.13 (0.0, 0.26)	0.33 (0.29, 0.74)	0.002
Unstimulated submandibular, ml/min/gland	0.0 (0.0, 0.0)	0.0 (0.0, 0.3)	0.03
Stimulated submandibular, ml/min/gland	0.01 (0.0, 0.05)	0.20 (0.16, 0.27)	0.0001
Schirmer I test, mm/5 min	2.5 (1.5, 7.5)	6.5 (3.5, 21.5)	0.01
Fluorescein staining	5 (3, 7)	3 (1, 5)	0.04
ANA, median titer	640 (80, 1280)	0 (0, 80)	< 0.0001
Positive anti-SSA, %	74 (n = 29)	7 (n = 1)	< 0.0001
Positive anti-SSB, %	31 (n = 12)	0	0.02
Rheumatoid factor, IU/ml	53 (22, 208)	0 (0, 0)	0.0001
IgG, mg/dl	1550 (1170, 2020)	1030 (899, 1190)	0.0004
IgA, mg/dl	232 (165, 303)	215 (162, 276)	NS
IgM, mg/dl	124 (91, 193)	137 (72, 159)	NS
Total protein, g/dl	7.7 (7.3, 8.0)	7.3 (7.1, 7.5)	NS
ESR, mm/h	34 (16, 61)	18 (15, 41)	NS
Hormone replacement therapy, %	31 (n = 12)	47 (n = 7)	NS

* Diagnosis by San Diego criteria[10].

Table 2. Comparison of sex steroid hormones for cases with primary SS and controls. Data are median values (25th, 75th percentile).

Laboratory Test	Normal Range	Cases (pSS)*, n = 39	Controls, n = 15	p
Testosterone, ng/dl	20–80	30.5 (24, 36)	30.0 (13, 37)	NS
SHBG, nmol/l	20–130	85 (71, 105)	131 (58, 237)	NS
DHEA, ng/dl	160–800	220 (170, 290)	250 (130, 310)	NS
DHEA sulfate, µg/ml	< 3	0.52 (0, 0.86)	0.48 (0, 0.76)	NS
DHT, ng/dl	4–22	4.4 (1.4, 8)	0.0 (0.0, 5)	0.04
Androstenedione, ng/dl	20–310	70 (50, 110)	60 (30, 120)	NS
Estradiol, pg/ml	0–400	57.5 (15, 110)	59.0 (22, 117)	NS
Estrone, pg/ml	14–346	36.0 (21, 114)	75.0 (23, 148)	NS

* Diagnosis by San Diego criteria[10]. SHBG: sex hormone binding globulin.

0.02) and a negative correlation between DHEA and ANA (r = –0.42, p = 0.04). It is interesting that although HRT clearly affected SHBG levels, the correlation with anti-SSB remained when pSS patients not using HRT were analyzed separately. Similar to the results with all pSS patients, inconsistent findings were observed with androgens and measures of disease activity.

DISCUSSION

All values of sex steroid hormones for patients with pSS were within the normal range, while xerostomic controls had DHT concentrations below normal and SHBG concentrations above the normal range. When compared to cases of pSS, controls differed significantly only in having lower concentrations of DHT.

Androgens were not associated with any consistent pattern of disease activity. Negative correlations were found between SHBG and anti-SSA and between SHBG and anti-SSB, suggesting that a higher concentration of SHBG, and thus lower concentrations of free or bioavailable sex steroids, may be associated with a lower disease activity reflected by lower concentrations of these autoantibodies. We observed that higher concentrations of disease activity (sedimentation rate, serum protein, and focus score) were associated with higher concentrations of testosterone, while a negative correlation was found between DHT and CRP.

We hypothesized that patients with pSS would exhibit lower concentrations of testosterone than controls. We were surprised to find that all values for sex steroid hormones were within the normal range for patients with pSS. The presence of low DHT and high SHBG in the control group was also unexpected. This may be a spurious finding related to small numbers (n = 15) in the xerostomic control group. Due to the effect of HRT on SHBG and hormone concentra-

tions, we assessed whether xerostomic control patients using HRT had levels different from those not on HRT. A comparison showed that xerostomic controls using HRT (n = 7) had a higher median SHBG (230 nmol/l) than those not using HRT (n = 8; 88.5 nmol/l), although this difference did not achieve statistical significance (p = 0.08).

Higher concentrations of testosterone were associated with worse disease activity (ESR, serum protein, and focus score), while lower concentrations of DHT were associated with higher concentrations of CRP. This unexpected relationship with disease activity may point to the complexity of androgens in autoimmune disease or be a result of the relatively small sample size. These contradictory results between different androgens with ESR and CRP may be related to other factors. First, ESR and CRP may be poor predictors of disease activity and thus be unrelated to disease severity. Second, different androgens may play different inflammatory roles, which may account for the current apparent discrepancy. HRT may have also been a factor in associations between sex steroid hormones and disease activity indices. When patients using HRT were removed from the analysis, there was a loss of positive correlation between testosterone and ESR and between testosterone and serum protein. This finding may be related to decreased number of patients leading to nonsignificance (type II error), or HRT may play an additive role in certain disease manifestations of pSS.

It is unclear why non-pSS xerostomic patients had lower concentrations of DHT compared to pSS patients. A possible explanation may be that more non-pSS patients were taking HRT (47%) versus pSS patients (31%). The mean and median values for all androgens were lower in the non-pSS controls taking HRT compared to those not on HRT. Possibly as a result of the HRT effect, SHBG concentrations were higher in non-pSS patients taking HRT. Although DHT was not significantly different by nonparametric statistical analysis, this may represent a type II statistical error due to the small number of patients in the xerostomic control group. Another possible explanation of lower concentrations of DHT in the non-pSS xerostomic controls may be that although these patients did not meet the diagnostic criteria for pSS, they may fall into the spectrum of early SS. Low concentrations of androgens may be a manifestation of the early stages of pSS development.

A recent study by Valtysdottir, *et al* described lower DHEA-S levels in 10 pSS compared to 10 age-matched female controls[15]. We found no differences with DHEA-S in pSS compared to non-pSS controls. One possible explanation for the discrepancies may be related to HRT in our study. To more closely match exclusion criteria used by Valtysdottir, *et al*, we completed a secondary analysis of patients not taking HRT. We found similar results, with no difference in DHEA-S between pSS patients and controls. Additionally, both our study and the report by Valtysdottir, *et al* had small sample sizes (39 pSS to 15 xerostomic controls vs 10 pSS to 10 age-matched controls, respectively). Variations between these 2 studies may be related to bias introduced by a small sample size. It is possible that a comparison of DHEA-S in our study between pSS and a nonxerostomic control group may have led to results similar to Valtysdottir, *et al*.

A possible role for estrogens enhancing autoimmunity in pSS was suggested by Steinberg, *et al* in studies with NZB × NZW F_1 mice[5]. The group of oophorectomized mice showed more pronounced antibody production with estrogens. The results from these animal studies suggest that estrogens may be associated with the autoimmune response in pSS. Human studies have also suggested that estrogens may enhance disease activity[8]. Neither of these suggestions was confirmed by our study, which revealed normal estrogen concentrations and no relationship between estrogens and disease activity in pSS. Discrepant results found between different animal models and between human and animal models likely point to the complexity of hormones in pSS. It appears that hormones may have different effects in pSS depending on the site of action, with reports that some tissues respond differently to hormonal influence[6]. Additionally, important discrepancies in human and animal models could be related to differences in binding affinity to hormone receptors, which are not reflected by measures of endogenous hormone levels.

One difficulty identifying differences in estrogen values may be related to natural hormonal variations that occur with the human menstrual cycle. In our study, patients had to be at the same part of their menstrual cycle or in the same part of the hormone replacement cycle for those preparations allowing for withdrawal bleed. All patients with cyclical hormones (replacement or endogenous) had to be within 7 days of their menses. Thus, there were 2 scenarios: with cyclical hormones and with constant hormones. The hormone concentrations may have been normal in some patients because of the replacement.

Due to the possibility that cholesterol-lowering agents may alter sex steroid levels, we determined how many patients and controls were taking these medications. We found that few patients and controls (1/39 pSS and 2/15 xerostomic controls) were taking cholesterol-lowering agents. Median values of all sex steroid concentrations of pSS and xerostomic controls remained within the normal range when values of these patients were not included in the analysis. Comparisons of hormone levels between the 2 groups were not statistically different with removal of these subjects.

One limitation of our study, which may have precluded finding support for our hypotheses, was the small sample size of the xerostomic control group (n = 15). However, it is striking that for most sex steroid data, all subjects (controls and pSS) displayed values within the expected range for

healthy individuals. Another limitation is that no uniform agreement exists for the most appropriate measures of disease activity for pSS. Considering that most glandular, extraglandular, and serologic manifestations of SS remain stable over time, current measures of disease activity may not be good predictors of disease severity or progression.

Another possible limitation may be the methods of hormone measurement. More accurate measures of the total androgen pool in humans may be reflected by the serum concentration of conjugated DHT metabolites (e.g., androsterone-glucuronide, androstane-3α, 17ß-diol-glucuronide)[16,17]. Future studies comparing differences in these metabolites between pSS and controls, and investigating whether associations exist between measures of disease activity, may provide a clearer understanding of the role of hormones in pSS.

Finally, measures of sex steroid hormones at peripheral sites versus serum concentrations may be a more accurate reflection of the true physiologic effect of hormones. Local control of sex steroid hormones has been shown, where DHEA and DHEA-S secreted by the adrenal glands are converted into potent androgens (e.g., testosterone, DHT) and estrogens by steroidogenic enzymes in peripheral sites in response to local requirements[18]. Thus, levels of androgens and estrogens within labial salivary and lacrimal glands in SS may not be related to serologic measures of sex steroid hormones. In addition, alteration of sex steroid metabolism may be caused by proinflammatory cytokines. These considerations must be taken into account with the interpretation of our results and future studies that examine the relationship between sex steroid hormones and SS.

In summary, we detected no alteration in concentration of sex steroids in pSS, no clear relationship between the concentration of certain androgens and disease activity, and no relationship to estrogen concentration. Thus, no consistent relationship between sex steroid concentration and disease activity measures in pSS was found in this study. Our results suggest that either sex steroid hormones do not play a major role in the activity or progression of pSS, as previously suggested, or that serum concentration may not accurately reflect local hormonal influences at cellular and tissue levels. Further investigation is needed.

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